REMARKS

Regarding the Election of Species, Applicants affirm the election of the compound of Example 6. This is made without traverse, as there was no Restriction Requirement.

In the Office Action that was mailed November 25, 2002, Claims 36-53 were rejected under 35USC§112, first paragraph, as lacking enablement for "prevention" of the claimed diseases.

Claims 45-46, 48-49, and 55 were rejected under 35USC§112, first paragraph, as containing references to other patents.

Claims 45-46, 48-49, 53, and 55 were rejected under 35USC§112, first paragraph, for using the terms Syndrome X, ZD-4522, MCC-555, and KRP-297.

The following rejections were made under 35USC§112, second paragraph:

- Claims 17-18 were rejected for reciting what is not claimed.
- Claims 29-30 are indefinite for defining the substituents in formula Ia in accordance with Claim 1, although Claim 29 is written as an independent claim.
- Claims 33-34 are rejected because they recite the same species, by structure in Claim 33 and by name in Claim 34. The Examiner also objected to the format of Claim 33 because it was in the form of a Table.

The Examiner also provisionally rejected Claims 1-55 under 35USC§101 (same invention double patenting) over US Application No. 09/961,841. The Examiner suggested that the double patenting rejection can be overcome by filing a terminal disclaimer.

Finally, the Examiner recommended four additional limitations, discussed below to make the claims patentable.

Response to Rejections

These rejections have all been addressed and are discussed below.

Regarding the 35USC§112, first paragraph, rejections, the claims have been amended so that prevention is no longer claimed. Citations of patents in the claims have been deleted, as these citations in the claims are extraneous. Words such as "including" and "such as" have been deleted from the claims.

Claims 49 and 53 have been amended by substituting the USAN name "rosuvastatin" for "ZD-4522". Two pages from the list of new USAN names from the American Medical Association Website for 2001 and 2002 are included herewith. The two pages show the USAN name and structure for ZD-4522 and MCC-555. The research codes ZD-4522 and MCC-555 were well known to researchers in the field when the application was filed. MCC-455 is not used in the claims but is recited in the specification.

Applicants traverse the rejection of Claims 36-37 for using the the expression "Syndrome X." Syndrome X is a description of a combination of metabolic abnormalities that accompany insulin resistance. The expression is widely used by physicians and researchers who deal with Type 2 diabetes. It was first used in a publication by Gerald M. Reaven, which is enclosed and is cited on attached form PTO 1449. Note that the term "Syndrome X" is used in the heading of Table 1 on page 1605 of the publication.

Applicants are also enclosing a publication disclosing the structure of KRP-297. The term is not used in the claims, but is still used in the description. The structure of KRP-297 is shown on page 1842 of the publication by Koji Murakami, which is enclosed with this response and is cited on Form 1449.

Claim 33 has been cancelled and rewritten as New 56. New Claim 56 is written as a Markush claim, which conforms with US Practice. Claim 34 has been cancelled, so that the question of whether it is the same as claim 33 is moot.

Claims 17 and 18 were amended so that the negative claim language is written in the form of a proviso in each claim. This is supported by the definition of R^4 in Claim 1. The use of provisoes is acceptable in US Practice.

With respect to the double patenting rejection, the claims do not meet the standards of same invention double patenting. The definitions of R^4 in the two applications define different groups of substituents. The two definitions of R^4 are mutually exclusive. The applications do not claim the same subject matter or overlapping subject matter. They therefore do not claim the same invention.

Terminal disclaimers can be filed in a situation where there is obviousness type double patenting. It does not appear that the mutually exclusive claims of the two applications satisfy the requirements of obviousness type double patenting. Therefore, it is believed that a terminal disclaimer is not needed.

With respect to the other four suggestions for making the claims patentable, the following comments are provided;

(1) In order to expedite prosecution, the alternate definition of R^1, where R^1 forms a cyclopropane ring, has been deleted.

(2) The examiner has stated that there can be no further heterocyclic substituents. No rationale has been given for this requirement. Nevertheless, to expedite prosecution, the class of heterocycles defined in the application as "Hetaryl" has been deleted. The heterocyclic substituents "Hetcyc" and "Benzoheterocycle" were not deleted, as these are needed to claim all of the compounds that were synthesized. Examples 1-3 include benzisoxazoles in the structure, which are claimed through the alternative definition of R^4 in Claim 1. Example 4 has a benzisoxazole substituent, which is a Benzoheterocycle substituent. Examples 13 and 28 have heterocyclic substituents, which are within the definition of Hetcyc. Therefore, if the suggestion that should they be deleted is based on enablement, the use of these substitutents in the claims is well supported by the examples.

(3) "No further substituent at position R^1" is not understood. The definition of R^1 is supported by numerous examples of various substituents. There is only one R^1 position.

(4) Definitions of most substituents in the application other than R^4 do not include heterocycles. The heterocycles in R^4 are well supported by the examples and are necessary to claim applicants invention.

It is respectfully submitted that the claims are in condition for allowance. Such action is earnestly solicited. If the examiner wishes to discuss any matter relating to this application, the examiner is invited to telephone the undersigned attorney.

Respectfully submitted,

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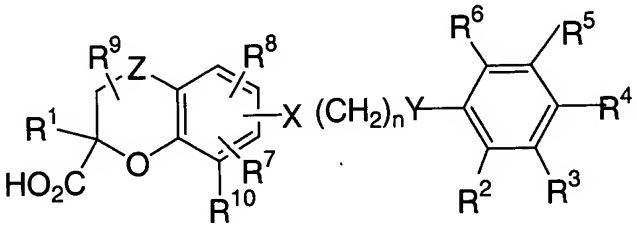
AMENDED CLAIMS SHOWING CHANGES

1. (Amended) A compound having the formula I:



I

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

Z is selected from the group consisting of CH_2 and C=O;

R^1 is selected from the group consisting of H, -OH, C_{1-7}alkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, -OC_{1-3}alkyl, -OC_{2-3}alkenyl, -OC_{2-3}alkynyl, F, Br, Cl, and Ar, wherein alkyl, alkenyl, alkynyl, -Oalkyl, -Oalkenyl and -Oalkynyl are linear or branched and are optionally substituted with (a) 1-7 halogen atoms, (b) 1-3 groups independently selected from (i) -OC_{1-3}alkyl, which is optionally substituted with 1-5 halogen atoms, and (ii) phenyl, which is optionally substituted with 1-3 groups independently selected from halogen, C_{1-5}alkyl and -OC_{1-3}alkyl, said C_{1-5}alkyl and -OC_{1-3}alkyl being linear or branched and optionally substituted with 1-5 halogens, or (c) a mixture of (a) and (b); ~~or alternatively, R^1 is a group $-CR^{11}R^{12}-$ which bridges between the carbon to which R^1 is attached in Figure I and the adjacent carbon on the heterocyclic ring, yielding a cyclopropane ring;~~

~~R^{11} and R^{12} are independently selected from the group consisting of hydrogen, halogen, C_{1-5}alkyl, C_{2-5}alkenyl, C_{2-5}alkynyl, -OC_{1-3}alkyl, -OC_{2-3}alkenyl, -OC_{2-3}alkynyl, -CO_2H, -CO_2C_{1-5}alkyl, -CO_2C_{2-5}alkenyl, -CO_2C_{2-5}alkynyl, and phenyl, where alkyl, alkenyl, alkynyl, -Oalkyl, -Oalkenyl, -Oalkynyl -CO_2alkyl, -CO_2alkenyl, and -CO_2alkynyl are linear or branched and are optionally substituted with (a) 1-5 halogens, (b) 1-3 substituents independently selected from -OCH_3 and -OCF_3, or (c) a mixture thereof, and phenyl is optionally substituted with 1-3 groups independently~~

~~selected from halogen, C_{1-5}alkyl, and $-OC_{1-3}$alkyl, wherein C_{1-5}alkyl and $-OC_{1-3}$alkyl are linear or branched and are optionally substituted with 1-5 halogens;~~

Ar is ~~selected from the group consisting of~~ Aryl, ~~Hetcyc, Hetaryl, and Benzoheterocycle, wherein~~ Aryl is~~, Hetcyc, Hetaryl, and Benzoheterocycle are~~ in each instance optionally substituted with 1-5 substituents independently selected from (a) halogen, (b) C_{1-5}alkyl, (c) C_{2-5}alkenyl, (d) C_{2-5}alkynyl, (e) $-OC_{1-5}$alkyl, (f) $-OC_{2-5}$alkenyl, (g) $-OC_{2-5}$alkynyl, (h) $-SO_xC_{1-5}$alkyl, (i) $-SO_xNR^aR^b$, (j) $-SO_x$phenyl, (k) $-C(O)C_{1-3}$alkyl, and (l) $-C(O)NR^aR^b$, wherein in each instance, each alkyl, alkenyl and alkynyl is linear or branched and is optionally substituted with (a) 1-5 halogen atoms, (b) 1-2 groups independently selected from $-OC_{1-3}$alkyl, which is linear or branched and is optionally substituted with 1-5 halogens, or (c) a mixture thereof, and wherein phenyl is optionally substituted with 1-3 substituents independently selected from halogen, C_{1-3}alkyl, and C_{1-3}alkoxy, wherein C_{1-3}alkyl and C_{1-3}alkoxy are linear or branched and are optionally substituted with 1-5 halogens;~~, and wherein Hetcyc and Benzoheterocycle may each optionally have a C_{3-6}-spiro-cycloalkyl substituent on the ring on a carbon atom that can have gem-disubstitution, wherein the spiro-cycloalkyl group is optionally substituted with 1-2 groups independently selected from methyl, trifluoromethyl, methoxy, trifluoromethoxy and halogen;~~

x is selected from 0, 1 and 2;

Aryl is a carbocyclic 6-10 membered monocyclic or bicyclic aromatic ring system;

Hetcyc is a 5- or 6-membered saturated or partly saturated monocyclic heterocycle having 1-4 heteroatoms independently selected from N, S and O in the perimeter of the ring, wherein N may optionally be NR^a and S may optionally be SO or SO_2;

~~Hetaryl is a 5- or 6-membered heteroaromatic ring having 1-4 heteroatoms independently selected from O, S, and N in the perimeter of the ring, where N may optionally be NR^a, and S may optionally be SO or SO_2;~~

Benzoheterocycle comprises a 5 or 6-membered heterocyclic ring which may be saturated, partly unsaturated or aromatic, and a benzene ring, wherein said heterocyclic ring and said

benzene ring are fused together, wherein said heterocyclic ring comprises 1-3 heteroatoms independently selected from O, S, and N in the perimeter of the ring, where N may optionally be NR^a, and S may optionally be SO or SO_2;

R^a and R^b are independently selected from the group consisting of H, C_{1-5}alkyl, C_{2-5}alkenyl, C_{2-5}alkynyl, -C(O)C_{1-5}alkyl, -C(O)C_{2-5}alkenyl, -C(O)C_{2-5}alkynyl, SO_xC_{1-5}alkyl, SO_xphenyl, $SOxNR^dR^e$, -C(O)NR^dR^e, halogen, and phenyl, wherein in all instances, alkyl, alkenyl, and alkynyl are linear or branched and are optionally substituted with (a) 1-5 halogen atoms, (b) 1-3 groups independently selected from $-OCH_3$, $-OCF_3$ and phenyl, or (c) a mixture thereof, wherein phenyl in all occurrences is optionally substituted with 1-3 substituents independently selected from halogen, C_{1-3}alkyl, and C_{1-3}alkoxy, said C_{1-3}alkyl and C_{1-3}alkoxy being linear or branched and optionally substituted with 1-5 halogens;

R^d and R^e are independently selected from H, C_{1-5}alkyl, C_{2-5}alkenyl, C_{2-5}alkynyl, and phenyl, wherein said alkyl, alkenyl, and alkynyl are linear or branched and are optionally substituted with (a) 1-5 halogen atoms, (b) 1-3 groups independently selected from $-OCH_3$, $-OCF_3$ and phenyl, or (c) a mixture thereof, wherein phenyl in all occurrences is optionally substituted with 1-3 substituents independently selected from halogen, C_{1-3}alkyl, and C_{1-3}alkoxy, said C_{1-3}alkyl and C_{1-3}alkoxy being linear or branched and optionally substituted with 1-5 halogens;

X and Y are independently selected from the group consisting of O, S, SO, SO_2, NR^a and CH_2;

n is an integer from 1-6;

R^2, R^3, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, C_{1-7}alkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, -OH, -OC_{1-5}alkyl, -OC_{2-5}alkenyl, -OC_{2-5}alkynyl, -C(O)C_{1-5}alkyl, -C(O)C_{2-5}alkenyl, -C(O)C_{2-5}alkynyl, -C(O)OC_{1-5}alkyl, -C(O)OC_{2-5}alkenyl, -C(O)OC_{2-5}alkynyl, -OC(O)C_{1-5}alkyl, -OC(O)C_{2-5}alkenyl, -OC(O)C_{2-5}alkynyl, Ar, -OAr, -C(O)Ar, -C(O)OAr, -OC(O)Ar, C_{3-8}Cycloalkyl, -OC_{3-8}Cycloalkyl, $-SO_xC_{1-5}$alkyl, $-SO_xNR^aR^b$, $-SO_xAr$, and -C(O)NR^aR^b, wherein in each instance, each alkyl, alkenyl, and alkynyl is linear or branched and is optionally substituted with (a) 1-5 halogen atoms, (b) 1-2 groups independently

selected from -OC_{1-3}alkyl groups which are linear or branched and are optionally substituted with 1-5 halogens, (c) 1 group Ar or C_{3-6}Cycloalkyl, or (d) a mixture of more than one of (a), (b) and (c);

R^4 is selected from the group consisting of Benzoheterocycle, C_{3-8}Cycloalkyl, Hetcyc, -OC_{3-8}Cycloalkyl and R^c, with the proviso that if R^4 is R^c, then either (1) R^1 is not H, and no more than one of R^2 , R^6, and R^{10} is alkyl, or (2) R^2 is Cl, Br or F, and R10 is not alkyl;

wherein Benzoheterocycle, C_{3-8}Cycloalkyl, Hetcyc and -OC_{3-8}Cycloalkyl are each optionally substituted with 1-3 groups independently selected from halogen, C_{1-5}alkyl, C_{2-5}alkenyl , C_{2-5}alkynyl , -OC_{1-5}alkyl, -OC_{2-5}alkenyl, -OC_{2-5}alkynyl, C_{3-8}Cycloalkyl, -SO_xC_{1-5}alkyl, -$SO_xNR^aR^b$,–SO_xphenyl, C(O)C_{1-3}alkyl and -C(O)NR^aR^b, wherein in all instances, said C_{1-5}alkyl, C_{2-5}alkenyl , and C_{2-5}alkynyl groups are linear or branched and are optionally substituted with 1-3 halogens, and wherein Hetcyc, -OC_{3-8}Cycloalkyl and C_{3-8}Cycloalkyl may optionally have a C_{3-6}-spiro-cycloalkyl substituent on the ring where gem-disubstitution of a ring carbon is possible, wherein the spiro-cycloalkyl group is optionally substituted with 1-2 groups independently selected from methyl, trifluoromethyl, methoxy, trifluoromethoxy and halogen;

wherein R^c is selected from the group consisting of halogen, -OH, -OSO_2C_{1-8}alkyl, -OSO_2C_{3-8}Cycloalkyl, -OSO_2Ar, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, -OC_{1-8}alkyl, -OC_{2-8}alkenyl, -OC_{2-8}alkynyl, and Aryl, wherein said -OSO_2C_{1-8}alkyl, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, -OC_{1-8}alkyl, -OC_{2-8}alkenyl, and -OC_{2-8}alkynyl are linear or branched, and are optionally substituted with (a) 1-5 halogens, (b) 1-2 groups independently selected from -OC_{1-3}alkyl, which are linear or branched and which are optionally substituted with 1-5 halogens, (c) 1 group selected from Aryl and C_{3-8}Cycloalkyl, or (d) a mixture of one or more of (a), (b) and (c), and Aryl and C_{3-8}Cycloalkyl are each optionally substituted as defined under Ar for Aryl and R^4 for C_{3-8}Cycloalkyl;

or alternatively R^4 and the adjacent substituent R^3 or R^5 may be connected to form a 5- or 6-membered heterocyclic ring that may be saturated, partly unsaturated or aromatic fused to the benzene ring, wherein the 5- or 6-membered fused ring comprises 1-3 heteroatoms independently selected from O, S, and N, where N may optionally be NR^a and S may optionally be SO or SO_2, said fused ring optionally also comprising 1-2 C=O groups in the perimeter of the ring, wherein said 5- or 6-membered heterocyclic fused ring is optionally substituted with 1-2 groups independently selected from R^3.

17. (Amended) A compound as recited in Claim 1, wherein R^4 is R^c, R^1 is selected from the group consisting of -OH, C_{1-7}alkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, -OC_{1-3}alkyl, -OC_{2-3}alkenyl, -OC_{2-3}alkynyl, F, Br, Cl, and Ar, wherein alkyl, alkenyl, alkynyl, -Oalkyl, -Oalkenyl and -Oalkynyl are linear or branched and are optionally substituted with (a) 1-7 halogen atoms, (b) 1-3 groups independently selected from (i) -OC_{1-3}alkyl, which is optionally substituted with 1-5 halogen atoms, and (ii) phenyl, which is optionally substituted with 1-3 groups independently selected from halogen, C_{1-5}alkyl and -OC_{1-3}alkyl, said C_{1-5}alkyl and -OC_{1-3}alkyl being linear or branched and optionally substituted with 1-5 halogens, or (c) a mixture of (a) and (b); ~~is not H or -$CR^{11}R^{12}$-, and~~ with the proviso that no more than one of R^2, R^6, and R^{10} is alkyl.

18. (Amended) A compound as recited in Claim 1, wherein R^4 is R^c, and R^2 is Cl, Br or F, ~~and~~ with the proviso that R^{10} is not alkyl.

36. (Amended) A method for treating~~,~~ or controlling~~, or preventing~~ non-insulin dependent (Type 2) diabetes mellitus in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

37. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ hyperglycemia in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

38. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ lipid disorders, hyperlipidemia, or low HDL in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

39. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ obesity in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

40. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ hypercholesterolemia in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

41. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ hypertriglyceridemia in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

42. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ dyslipidemia and/or low HDL cholesterol in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

43. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ atherosclerosis in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

44. (Amended) A method for treating~~,~~ or controlling ~~or preventing~~ cachexia in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 1.

45. (Amended) A method of treating~~,~~ or controlling ~~or preventing~~ one or more diseases, disorders, or conditions selected from the group consisting of (1) non-insulin dependent diabetes mellitus (NIDDM), (2) hyperglycemia, (3) impaired glucose tolerance, (4) insulin resistance, (5) obesity, (6) lipid disorders, (7) dyslipidemia, (8) hyperlipidemia, (9) hypertriglyceridemia, (10) hypercholesterolemia, (11) low HDL levels, (12) high LDL levels, (13) atherosclerosis and its sequelae, (14) vascular restenosis, (15) irritable bowel syndrome, (16) inflammatory bowel disease, including Crohn's disease and ulcerative colitis, (17) other inflammatory conditions, (18) pancreatitis, (19) abdominal obesity, (20) neurodegenerative disease, (21) retinopathy, (22) neoplastic conditions, (23) adipose cell tumors, (24) adipose cell carcinomas, such as liposarcoma, (25) prostate cancer and other cancers, including gastric, breast, bladder and colon cancers, (26) angiogenesis, (27) Alzheimer's disease, (28) psoriasis, (29) acne vulgaris, (30 skin diseases modulated by PPAR, (31) high blood pressure, (32) Syndrome X, (33) ovarian hyperandrogenism (polycystic ovarian syndrome), and other disorders where insulin resistance is a component, said method comprising the administration of an effective amount of a compound of Claim 1.

46. (Amended) A method of treating~~,~~ or controlling ~~or preventing~~ one or more diseases, disorders, or conditions selected from the group consisting of (1) diabetes mellitus, ~~and especially~~ and non-insulin dependent diabetes mellitus (NIDDM), (2) hyperglycemia, (3) impaired glucose tolerance, (4) insulin resistance, (5) obesity, (6) lipid disorders, (7) dyslipidemia, (8) hyperlipidemia, (9) hypertriglyceridemia, (10) hypercholesterolemia, (11) low HDL levels, (12) high LDL levels, (13) atherosclerosis and its sequelae, (14) vascular restenosis, (15) irritable bowel syndrome, (16) inflamatory bowel disease, including Crohn's disease and ulcerative colitis, (17) other inflammatory conditions, (18) pancreatitis, (19) abdominal obesity, (20) neurodegenerative disease, (21) retinopathy, (22) neoplastic conditions, (23) adipose cell tumors, (24) adipose cell carcinomas, such as liposarcoma, (25) prostate cancer and other cancers, including gastric, breast, bladder and colon cancers, (26) angiogenesis, (27) Alzheimer's disease, (28) psoriasis, (29) acne vulgaris, (30) skin diseases modulated by PPAR, (31) high blood pressure, (32) Syndrome X, (33) ovarian hyperandrogenism (polycystic ovarian syndrome), and other disorders where insulin resistance is a component, said method comprising the administration of an effective amount of a compound of Claim 1, and an effective amount of one or more other compounds selected from the group consisting of:

(a) insulin sensitizers~~;~~ ~~including~~ (i) PPARγ agonists ~~such as the glitazones (e.g. troglitazone, pioglitazone, englitazone, MCC-555, rosiglitazone, and the like), and compounds disclosed in WO97/27857, 97/28115, 97/28137 and 97/27847~~; (ii) biguanides ~~such as metformin and phenformin~~; (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors~~;~~; ~~and~~ (iv) dipeptidyl peptidase IV inhibitors;

(b) insulin or insulin mimetics;

(c) sulfonylureas ~~such as tolbutamide and glipizide, or related materials~~;

(d) α-glucosidase inhibitors ~~(such as acarbose)~~;

(e) cholesterol lowering agents selected from the group consisting of ~~such as~~ (i) HMG-CoA reductase inhibitors ~~(lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rivastatin, itavastatin, ZD-4522 and other statins)~~, (ii) sequestrants ~~(cholestyramine, colestipol, and dialkylaminoalkyl derivatives of a cross-linked dextran)~~, (iii) nicotinyl alcohol, nicotinic acid or a salt

thereof, (iv) PPARα agonists ~~such as fibric acid derivatives (clofibrate, fenofibrate and bezafibrate) or gemfibrozil~~, (v) PPARα/γdual agonists, ~~such as KRP-297,~~ (vi) inhibitors of cholesterol absorption, ~~such as for example ezetimibe,~~ (vii) acyl CoA:cholesterol acyltransferase inhibitors, ~~such as for example avasimibe,~~ and (viii) anti-oxidants~~, such as probucol~~;

(f) PPARδ agonists ~~such as those disclosed in WO97/28149~~;

(g) antiobesity compounds (anorectics) ~~such as fenfluramine, dexfenfluramine, phentermine, sibutramine, mazindol, orlistat, lipase inhibitors, neuropeptide Y5 inhibitors, and β3 adrenergic receptor agonists~~;

(h) an ileal bile acid transporter inhibitor; and

(i) anti-inflammatory agents. ~~intended for use in inflammatory conditions such as aspirin, non-steroidal anti-inflammatory drugs, glucocorticoids, azulfidine, and cyclo-oxygenase 2 selective inhibitors.~~

47. (Amended) A method for the treatment~~,~~ or control~~, or prevention~~ of one or more conditions selected from hypercholesterolemia, atherosclerosis, low HDL levels, high LDL levels, hyperlipidemia, hypertriglyceridemia, and dyslipidemia, which method comprises administering to a mammalian patient in need of such treatment a therapeutically effective amount of a compound of Claim 1 and a therapeutically effective amount of an HMG-CoA reductase inhibitor.

49. (Amended) The method as recited in Claim 48, wherein the statin is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, itavastatin, ~~ZD-4522~~ rosuvastatin and rivastatin.

50. (Amended) A method for the treatment~~,~~ or control~~, or prevention~~ of one or more conditions selected from inflammatory conditions, inflammatory bowel disease, Crohn's disease, and ulcerative colitis, which method comprises administering to a mammalian patient in need of such treatment a therapeutically effective amount of a compound according to Claim 1.

51. (Amended) A method for treating~~,~~ or preventing ~~or controlling~~ atherosclerosis in a mammalian patient in need of such treatment comprising the administration to said patient of an effective amount of a compound of Claim 1 and an effective amount of an HMG-CoA reductase inhibitor.

53. (Amended) The method as recited in Claim 52, wherein the statin is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, itavastatin, ~~ZD-4522~~ rosuvastatin and rivastatin.

54. (Amended) A pharmaceutical composition ~~for the treatment, prevention or control of atherosclerosis,~~ comprising: (1) a compound according to Claim 1, (2) an HMG-CoA reductase inhibitor, and (3) a pharmaceutically acceptable carrier.

55. (Amended) A pharmaceutical composition comprising (1) a compound according to Claim 1, (2) one or more compounds selected from the group consisting of :

(a) insulin sensitizers; ~~including~~ (i) PPARγ agonists ~~such as the glitazones (e.g. troglitazone, pioglitazone, englitazone, MCC-555, rosiglitazone, and the like), and compounds disclosed in WO97/27857, 97/28115, 97/28137 and 97/27847~~; (ii) biguanides ~~such as metformin and phenformin~~; (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors~~,~~; and (iv) dipeptidyl peptidase IV (DP-IV) inhibitors;

(b) insulin or insulin mimetics;

(c) sulfonylureas ~~such as tolbutamide and glipizide, or related materials~~;

(d) α-glucosidase inhibitors ~~(such as acarbose)~~;

(e) cholesterol lowering agents selected from the group consisting of ~~such as~~ (i) HMG-CoA reductase inhibitors ~~(lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rivastatin, itavastatin, ZD-4522 and other statins)~~, (ii) sequestrants ~~(cholestyramine, colestipol, and dialkylaminoalkyl derivatives of a cross-linked dextran)~~, (iii) nicotinyl alcohol, nicotinic acid or a salt thereof, (iv) PPARα agonists ~~such as fibric acid derivatives (clofibrate, fenofibrate and bezafibrate) or gemfibrozil~~, (v) PPARα/γdual agonists, ~~such as KRP-297,~~ (vi) inhibitors of cholesterol absorption, ~~such as for example ezetimibe,~~ (vii) acyl CoA:cholesterol acyltransferase inhibitors, ~~such as for example avasimibe,~~ and (viii) anti-oxidants~~, such as probucol~~;

(f) PPARδ agonists ~~such as those disclosed in WO97/28149~~;

(g) antiobesity compounds (anorectics) ~~such as fenfluramine, dexfenfluramine, phentermine, sibutramine, mazindol, orlistat, lipase inhibitors, neuropeptide Y5 inhibitors, and β3 adrenergic receptor agonists~~;

(h) an ileal bile acid transporter inhibitor; and

(i) anti-inflammantory agents ~~intended for use in inflammatory conditions such as aspirin, non-steroidal anti-inflammatory drugs, glucocorticoids, azulfidine, and cyclo-oxygenase 2 selective inhibitors~~; and (3) a pharmaceutically acceptable carrier.

A Novel Insulin Sensitizer Acts as a Coligand for Peroxisome Proliferator-Activated Receptor-α (PPAR-α) and PPAR-γ

Effect of PPAR-α Activation on Abnormal Lipid Metabolism in Liver of Zucker Fatty Rats

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We investigated the biological activity of a novel thiazolidinedione (TZD) derivative, KRP-297, and the molecular basis of this activity. When administered to obese Zucker fatty rats (obese rats) at 10 mg/kg for 2 weeks, KRP-297, unlike BRL-49,653, restored reduced lipid oxidation, that is, CO_2 and ketone body production from [^{14}C]palmitic acid, in the liver by 39% ($P < 0.05$) and 57% ($P < 0.01$), respectively. KRP-297 was also significantly more effective than BRL-49,653 in the inhibition of enhanced lipogenesis and triglyceride accumulation in the liver. To understand the molecular basis of the biological effects of KRP-297, we examined the effect on peroxisome proliferator-activated receptor (PPAR) isoforms, which may play key roles in lipid metabolism. Unlike classical TZD derivatives, KRP-297 activated both PPAR-α and PPAR-γ, with median effective concentrations of 1.0 and 0.8 μmol/l, respectively. Moreover, radiolabeled [^{3}H]KRP-297 bound directly to PPAR-α and PPAR-γ with dissociation constants of 228 and 326 nmol/l, respectively. Concomitantly, KRP-297, but not BRL-49,653, increased the mRNA and the activity (1.5-fold [$P < 0.01$] and 1.8-fold [$P < 0.05$], respectively) of acyl-CoA oxidase, which has been reported to be regulated by PPAR-α, in the liver. By contrast, KRP-297 ($P < 0.05$) was less potent than BRL-49,653 ($P < 0.01$) in inducing the PPAR-γ–regulated aP2 gene mRNA expression in the adipose tissues. These results suggest that PPAR-α agonism has a protective effect against abnormal lipid metabolism in liver of obese rats. *Diabetes* **47:1841–1847, 1998**

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ACO, acyl-CoA oxidase; DMEM, Dulbecco's modified Eagle's medium; β-gal, β-galactosidase; K_d, dissociation constant; obese rat, obese Zucker fatty rat; LBD, ligand-binding domain; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-response element; TG, triglyceride; TZD, thiazolidinedione.

Peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ are orphan members of the nuclear receptor superfamily, which have recently been proposed to play key roles in lipid and carbohydrate homeostasis (1). PPAR-α is predominantly expressed in several tissues that have high lipid catabolism activity, such as the liver (2,3). PPAR-α is known to be activated by a variety of structurally diverse compounds, such as hypolipidemic drugs of the fibrate class (4). There is no apparent structural similarity among PPAR-α activators other than the presence of a hydrophobic backbone linked to a carboxylic acid group or a group that can be readily metabolized to a carboxylic acid group. Although it is now established that these chemicals can activate PPAR-α, several possible direct or indirect mechanisms have been proposed (5–8). On the other hand, antidiabetic agents such as thiazolidinedione (TZD) derivatives have been reported to bind directly to the PPAR-γ expressed abundantly in adipose tissues (2,3,9,10).

Elevated circulating lipids have been proposed to be a cause of the development of insulin resistance associated with obesity (11). A recent insulin clamp study indicated that circulating lipids bring about insulin resistance in muscle by inhibiting glucose transport and/or phosphorylation (12). Moreover, elevated circulating lipids appear to enhance gluconeogenesis in the liver and impair compensatory insulin secretion from pancreatic β-cells for insulin resistance (13,14). Obese Zucker fatty rats (obese rats) display hyperlipidemia, hyperinsulinemia, and mild hyperglycemia and are widely used as an excellent animal model of human NIDDM associated with obesity. The liver of these rats has been reported to exhibit enhanced lipogenesis and reduced lipid oxidation (15,16). The functional peroxisome proliferator-response elements (PPREs) have been identified in genes encoding several enzymes in the catabolic pathway of lipid metabolism in the liver (17–19). Nevertheless, the role of PPARs in abnormal lipid metabolism in liver of obese rats remains unclear.

We show that a novel TZD derivative synthesized in our laboratory, KRP-297, improves abnormal lipid metabolism in liver of obese rats in addition to showing hypoglycemic, hypoinsulinemic, and hypolipidemic actions. To identify the molecular basis of these biological effects, we investigated the

effect of KRP-297 on PPAR isoforms. In this study, we show that KRP-297 activated both PPAR-α and PPAR-γ and that it is a novel coligand of these PPAR isoforms. These results suggest that PPAR-α agonism has a protective effect against abnormal lipid metabolism in liver of obese rats.

RESEARCH DESIGN AND METHODS

Chemicals. KRP-297 and other TZD derivatives were synthesized by Kyorin (Tochigi, Japan). WY-14.643 was purchased from BIOMOL (Plymouth Meeting, PA). Bezafibrate was supplied by Kissei (Matsumoto City, Japan). [^{3}H]KRP-297 was synthesized at Amersham Life Science (Amersham, U.K.). All compounds were dissolved in DMSO, and the final DMSO concentrations were kept <0.1% in all assays.

Animals. Male obese rats and lean littermates (+/?) were obtained from the Jackson Laboratory (Bar Harbor, ME). All rats were given standard rat diet (OA-2; Japan Crea) and tap water ad libitum. All institutional guidelines for animal care and use were applied in this study. Obese and lean rats (n = 5) were 9 weeks old at the start of drug administration. KRP-297 (10 mg/kg), BRL-49,653 (10 mg/kg), or vehicle (0.5% gum arabic solution) was administered orally for 2 weeks. At the end of the treatment period, plasma samples were collected. The liver and retroperitoneal adipose tissues were removed.

Lipid metabolism and enzymatic activity of acyl-CoA oxidase in liver. The measurements of [^{14}C]CO_2 and ketone body production from [1-^{14}C]palmitic acid and lipogenesis from [1-^{14}C]acetate were performed using liver slices, as described (20). Liver homogenates were extracted with an extract solution ($CHCl_3$:CH_3OH = 2:1), and the triglyceride (TG) content was then determined. The remainder of the liver was immediately frozen in liquid nitrogen and stored at −80°C until measurements of the enzymatic activity of acyl-CoA oxidase (ACO) were made. ACO activity in the light mitochondrial fraction of liver was measured by assay that was based on the H_2O_2-dependent oxidation of leuco-dichlorofluorescein (21,22).

Assays of plasma sample. Plasma glucose and free fatty acid levels were determined by glucose B-test and NEFA C-test (Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin and TG levels were measured by insulin immunoassay (Morinaga Institute of Biological Science, Yokohama, Japan) and determiner TG-S 555 (Kyowa Medex, Shizuoka, Japan). β-Hydroxybutyric acid levels were determined enzymatically.

Transactivation assay. A cDNA of the putative ligand-binding domain (LBD) encoding amino acids 167–468, 204–506, or 139–441 of human PPAR-α (23), PPAR-γ (24), or PPAR-δ (NUC-I) (25), respectively, was inserted into the pSG5 expression vector containing elements of both GAL4 (amino acids 1–147) and amino acids 1–76 of the glucocorticoid receptor. The chimeric expression plasmids (GAL4-hPPAR LBD), a GAL4-responsive luciferase reporter (UAS_Gx4-TK-LUC), the pRS expression plasmid of full-length cDNA of rat PPAR-α (26), and the luciferase reporter containing three copies of rat ACO PPRE (ACO-PPREx3-LUC) (17) were provided by Dr. S.A. Kliewer (Glaxo Wellcome, London, U.K.). CV-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% delipided fetal calf serum and antibiotics. CV-1 cells were transfected with receptor expression plasmid, luciferase reporter expression plasmid, and β-galactosidase (β-gal) expression plasmid. Transfection was carried out by Lipofectin (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's instructions. After transfection, cells were treated with the indicated compounds for 24 h, and cell extracts were then prepared and assayed for luciferase and β-gal activities. Luciferase activity was normalized using the β-gal activity as an internal standard.

Binding assay. Each LBD of PPAR-α and PPAR-γ was introduced into the pQE-30 bacterial expression vector (QIAGEN, Hilden, Germany). The expression of histidine-tagged PPAR-α and PPAR-γ in JM-109 was induced by the addition of isopropyl β-thiogalactopyranoside to the growth medium. Bacterial extracts were prepared using standard methods, and the fusion proteins were purified by elution through a nickel-ion agarose column. Binding assays were performed by incubating these fusion proteins (5 μg of protein) and [^{3}H]KRP-297 (specific activity, 27 Ci/mmol) at 25°C for 45 min in a buffer containing 10 mmol/l Tris (pH 8.0), 50 mmol/l KCl, and 10 mmol/l dithiothreitol. Competitors were added in a reaction as indicated in the figure legends. Bound [^{3}H]KRP-297 was immediately separated from free [^{3}H]KRP-297 on a 1-ml Sephadex G-25 spin column (Pharmacia, Uppsala, Sweden), which was equilibrated in 25 mmol/l Tris (pH 7.4), 75 mmol/l KCl, 15% glycerol, 0.05% Triton X-100, and 0.5 mmol/l EDTA. The radioactivity of the bound [^{3}H]KRP-297 fraction was determined by liquid scintillation counting.

Culture of hepatocytes. Rat hepatocytes were isolated by collagenase perfusion of liver from male Wistar rats (27). Hepatocytes were cultured in DMEM supplemented with 10% fetal calf serum, dexamethasone, insulin, and antibiotics. After 24 h, hepatocytes were treated with various concentrations of compounds or vehicle for 24 h.

Northern blotting. Total RNA from cells and tissue homogenates was isolated by ISOGEN (Nippon Gene, Toyama, Japan), and Northern blotting was then performed with the probes for rat ACO (Dr. T. Hashimoto), mouse aP2 cDNA (Dr. T. Kawada), or rat β-actin mRNA. Results were analyzed by quantitative scanning densitometry, and the amount of each mRNA was normalized to β-actin mRNA levels.

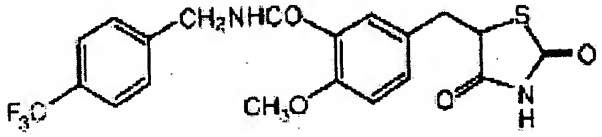
Statistical analysis. All results obtained from animal studies are presented as means ± SE. The significance of the difference in mean values between KRP-297- and BRL-49,653-treated obese rats, or between lean and obese control rats, was assessed using the unpaired Student's t test. The statistical significance between the obese control rats and the compound-treated obese rats was assessed by Dunnett's test.

RESULTS

Body weight, liver weight, and plasma parameters in obese rats. Figure 1 shows the chemical structure of KRP-297. When administered orally to obese rats at 10 mg/kg for 2 weeks, KRP-297 and BRL-49,653 had no significant effect on the body and liver weights of these rats (Table 1). KRP-297 and BRL-49,653 were effective in lowering plasma glucose, insulin, TG, and free fatty acid levels in obese rats. The plasma β-hydroxybutyric acid level in obese control rats was decreased to 32% ($P < 0.05$) of that in lean control rats. BRL-49,653 only slightly decreased plasma β-hydroxybutyric acid levels in obese rats (35%). KRP-297 showed no significant effect on plasma β-hydroxybutyric acid levels in obese rats.

Lipid metabolism in liver of obese rats. The effect of KRP-297 and BRL-49,653 on TG levels in liver of obese rats was examined (Fig. 2*A*). TG levels in obese control rats were threefold higher than those in lean control rats. KRP-297 and BRL-49,653 inhibited TG accumulation in liver of obese rats by 73% ($P < 0.01$) and 38% ($P < 0.05$), respectively. This inhibitory effect of KRP-297 on TG accumulation was significantly greater than that of BRL-49,653 ($P < 0.05$). The production of CO_2 and ketone body from [^{14}C]palmitic acid (lipid oxidation) in liver of obese control rats was, respectively, 69% ($P < 0.05$) and 59% ($P < 0.05$) lower than that in lean control rats (Fig. 2*B* and *C*). KRP-297 improved the reduced CO_2 and ketone body production in liver of obese rats by 39% ($P < 0.01$) and 57% ($P < 0.01$), respectively, whereas BRL-49,653 had no significant effect. The lipogenesis from [^{14}C]acetate in liver of obese control rats was approximately twofold greater than that in lean control rats ($P < 0.01$) (Fig. 2*D*). KRP-297 completely inhibited the enhanced lipogenesis in liver of obese rats ($P < 0.05$), whereas BRL-49,653 had only a minimal effect (NS).

Transactivation of PPAR isoforms. Because KRP-297 showed biological effects such as amelioration of hyperglycemia, hyperinsulinemia, and hyperlipidemia in obese rats, as well as abnormal lipid metabolism in the liver, we investigated the effect of KRP-297 on PPAR isoforms. KRP-297 activated GAL4-hPPAR-αLBD in a dose-dependent manner (Fig. 3*A*), producing a 16-fold activation at 10 μmol/l, while other TZD derivatives such as BRL-49,653, pioglitazone, and



KRP-297

FIG. 1. Chemical structure of KRP-297.

TABLE 1
Biochemical characteristics in rats treated with KRP-297 or BRL-49,653

Rat	Treatment	Body weight (g)	Liver weight (g)	Plasma levels: Glucose (mmol/l)	Insulin (pmol/l)	TG (mmol/l)	Free fatty acid (g/l)	β-Hydroxybutyric acid (μmol/l)
Obese	Vehicle	478 ± 15	23.2 ± 1.3	8.7 ± 0.6	817 ± 17	5.17 ± 1.13	0.31 ± 0.03	95 ± 10
Obese	KRP (10 mg/kg)	515 ± 13	20.9 ± 1.3	5.7 ± 0.2*	567 ± 67*	1.12 ± 0.10*	0.12 ± 0.01*	104 ± 6.0
Obese	BRL (10 mg/kg)	530 ± 19	23.6 ± 1.5	5.9 ± 0.2*	617 ± 33†	1.22 ± 0.25*	0.14 ± 0.03*	62 ± 16
Lean	Vehicle	299 ± 10	10.8 ± 0.5	6.7 ± 0.7	217 ± 50	0.88 ± 0.09	0.27 ± 0.03	297 ± 61

Data are means ± SE. For all groups, $n = 5$. *$P < 0.01$ vs. obese vehicle; †$P < 0.05$ vs. obese vehicle.

troglitazone failed to activate it significantly at 10 μmol/l. WY-14,643 and bezafibrate, which are known potent peroxisome proliferators, also exhibited a seven- and eightfold activation of GAL4-hPPAR-αLBD at 10 μmol/l. KRP-297 and other TZD derivatives activated GAL4-hPPAR-γLBD in a dose-dependent manner (Fig. 3*B*); KRP-297 produced a tenfold activation at 10 μmol/l, and BRL-49,653 was the most potent activator. No compound tested significantly activated GAL4-hPPAR-δ (NUC-I) LBD under the same conditions (Fig. 3*C*). The median effective concentrations of KRP-297 for the activation of hPPAR-α and hPPAR-γ were 1.0 and 0.8 μmol/l, respectively.

Binding assay for PPAR-α and PPAR-γ. To clarify whether the transactivation of PPAR-α and PPAR-γ by KRP-297 is due to direct binding of KRP-297 to these PPAR isoforms, a binding assay was performed using [^{3}H]KRP-297 and the purified histidine-tagged hPPAR-α or hPPAR-γ LBD (His-hPPARsLBD). Binding of [^{3}H]KRP-297 to His-hPPAR-αLBD was saturable and was effectively displaced by a 625-fold excess of unlabeled KRP-297 (Fig. 4*A*), showing that the binding was specific. The dissociation constant (K_d) of KRP-297 for His-hPPAR-αLBD was calculated to be 228 nmol/l from Scatchard's analysis (Fig. 4*B*). [^{3}H]KRP-297 also bound to His-hPPAR-γLBD, and its binding was effectively displaced by a 625-fold excess of unlabeled KRP-297 (Fig. 4*C*). The K_d of KRP-297 for His-hPPAR-γLBD was calculated to be 326 nmol/l (Fig. 4*D*).

We then studied the binding of other compounds to His-hPPAR-αLBD or His-hPPAR-γLBD using [^{3}H]KRP-297 as a ligand. His-hPPAR-αLBD (Fig. 5*A*) or His-hPPAR-γLBD (Fig. 5*B*) was incubated with 100 nmol/l [^{3}H]KRP-297 in the presence of a 100-fold excess (10 μmol/l) of unlabeled competitors. KRP-297 and WY-14,643 competed with [^{3}H]KRP-297 for the binding to His-hPPAR-αLBD at 97 and 72%, respectively, whereas BRL-49,653, pioglitazone, troglitazone, and bezafibrate showed little or no competition. On the other hand, KRP-297, BRL-49,653, pioglitazone, and troglitazone competed with [^{3}H]KRP-297 for the binding to His-hPPAR-γLBD at 72, 90, 74, and 69%, respectively, whereas WY-14,643 and bezafibrate showed no competition.

ACO mRNA expression and activity in liver of obese rats. ACO is a rate-limiting enzyme in peroxisomal lipid oxidation in the liver. Because KRP-297 showed restoration of reduced lipid oxidation in obese rats, we examined mRNA levels (Fig. 6*A*) and the activity (Fig. 6*B*) of ACO in liver of obese rats treated with KRP-297. KRP-297, but not BRL-49,653, increased ACO mRNA levels 1.5-fold ($P < 0.01$) in liver of obese rats. The ACO activity in liver of obese control rats was reduced to 53% ($P < 0.01$) of that in lean control rats. KRP-297 restored the reduced ACO activity in liver of obese rats ($P < 0.05$), but BRL-49,653 showed no effect.

Transactivation of rat PPAR-α and ACO mRNA in primary rat hepatocytes. To examine whether KRP-297 can directly transactivate the ACO gene, which is known to possess a PPRE in the promoter region, we examined the transactivation of rat PPAR-α (Fig. 7*A*) and the expression of ACO mRNA in primary rat hepatocytes (Fig. 7*B*). KRP-297 and WY-14,643 increased the reporter (ACO-PPREx3-LUC) activity in rat PPAR-α transfected CV-1 cells, whereas BRL-49,653 showed no effect. To determine whether KRP-297 can directly induce the expression of ACO mRNA, primary rat hepatocytes were treated with KRP-297, WY-14,643, and BRL-49,653 for 24 h. KRP-297 and WY-14,643 increased ACO mRNA levels in primary rat hepatocytes in a dose-dependent manner, whereas BRL-49,653 provided little increase.

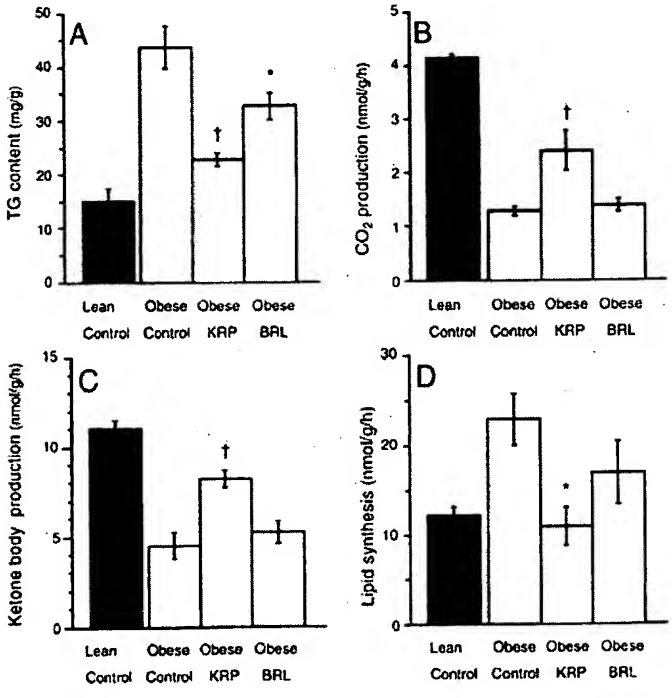


FIG. 2. The effects of KRP-297 and BRL-49,653 on abnormal lipid metabolism in liver of obese rats. Either KRP-297 (KRP) or BRL-49,653 (BRL) was administered orally to obese rats at a dosage of 10 mg/kg once a day for 2 weeks. TG content (*A*), [^{14}C]CO_2 (*B*), and ketone body (*C*) production from [^{14}C]palmitic acid, and lipid synthesis (*D*) from [^{14}C]acetate in liver of these rats were measured as described in METHODS. Data are means ± SE. *$P < 0.05$ vs. obese control rats; †$P < 0.01$ vs. obese control rats.

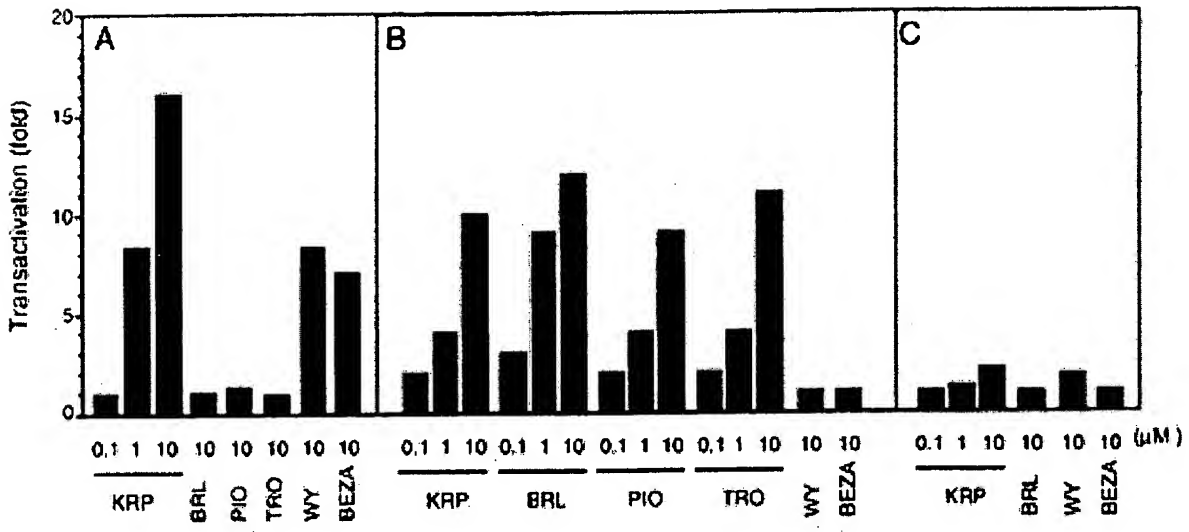


FIG. 3. Transactivation of PPAR isoforms by various compounds. GAL4-hPPAR-α (*A*), PPAR-γ (*B*), or PPAR-δ (NUC-I) (*C*) LBD plasmids were cotransfected into CV-1 cells with GAL4-responsive luciferase reporter plasmid and β-gal plasmid, and cells were then cultured in the presence of indicated concentrations of KRP-297 (KRP), BRL-49,653 (BRL), pioglitazone (PIO), troglitazone (TRO), WY-14,643 (WY), bezafibrate (BEZA), or vehicle (0.1% DMSO) control. Each luciferase activity was normalized by β-gal activity and represented as fold activation over the vehicle control.

aP2 mRNA expression in adipose tissues of obese rats. To examine the effect of KRP-297 on PPAR-γ activation in vivo, we determined aP2 mRNA levels in adipose tissues that may be upregulated by PPAR-γ (Table 2). The expression of aP2 mRNA in retroperitoneal adipose tissues was similar in lean and obese control rats. KRP-297 and BRL-49,653 showed increases of 8.3-fold ($P < 0.05$) and 11.2-fold ($P < 0.01$), respectively, in aP2 mRNA levels in retroperitoneal adipose tissues of obese rats.

DISCUSSION

In this study, we have shown that unlike classical TZD derivatives, KRP-297 is a novel coligand and activator of both PPAR-α and PPAR-γ. This study presents the first report of the effect of a coligand for PPAR-α and PPAR-γ in obese rats. These findings have demonstrated a beneficial effect of PPAR-α agonism on abnormal lipid metabolism in liver of obese rats.

In a transactivation assay, KRP-297 activated both PPAR-α and PPAR-γ with a similar potency. KRP-297 can bind directly to PPAR-α and PPAR-γ with K_d values of 228 and 326 nmol/l, respectively. The slight discrepancy between the concentrations that induce transactivation and the binding affinities might be explained by the ability of the compound to be transported across the membranes or to be bound by intracellular proteins. Our results showed that other TZD derivatives such as BRL-49,653, pioglitazone, and troglitazone were PPAR-γ–selective activators, whereas WY-14,643 and bezafibrate were PPAR-α–selective activators, which is consistent with the findings of previous reports (8,9). Although WY-14,643 effectively competed in [^{3}H]KRP-297 binding to PPAR-α, bezafibrate provided only slight competition. The dissociation between transcriptional activity and the binding affinity of bezafibrate may suggest the existence of indirect-activation mechanisms for PPAR-α (5–7). Thus, unlike classical activators for PPAR-α or PPAR-γ, KRP-297 was a novel coligand for PPAR-α and PPAR-γ. After completing this work, Kliewer et al. (28)

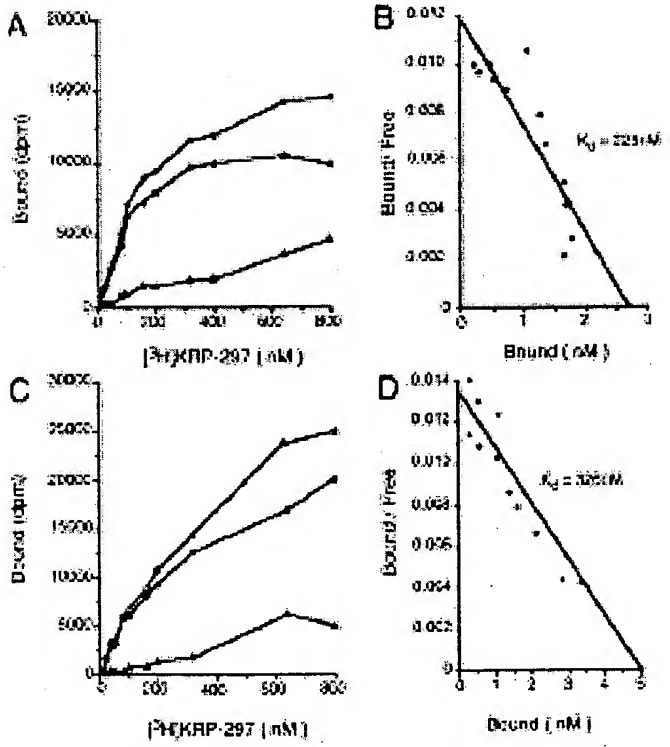


FIG. 4. KRP-297 is a high-affinity ligand for PPAR-α and PPAR-γ. His-hPPAR-α (*A*) or His-hPPAR-γ (*C*) LBD was incubated with various concentrations of [^{3}H]KRP-297 in the absence (total binding) (○) or presence (nonspecific binding) (▲) of a 625-fold excess of unlabeled KRP-297. Scatchard's analysis of [^{3}H]KRP-297 binding to His-hPPAR-α LBD (*B*) or to His-hPPAR-γ LBD (*D*) is based on the specific binding (●) of [^{3}H]KRP-297 in the data of *A* or *C*.

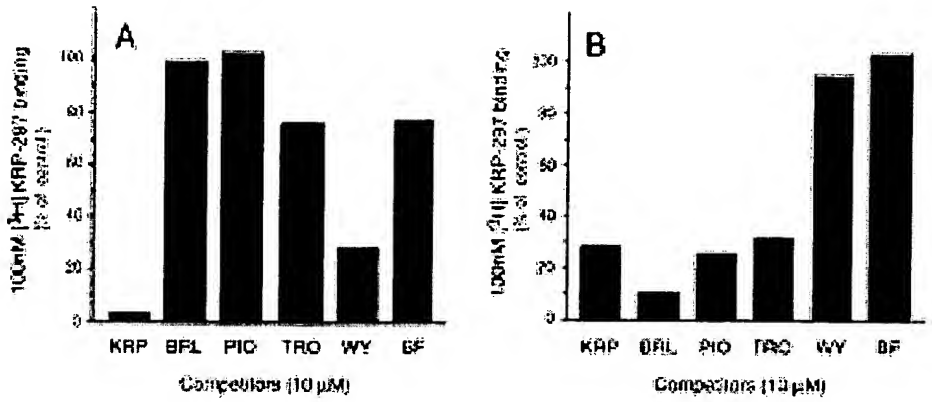


FIG. 5. Competition binding assay with [^{3}H]KRP-297. His-hPPAR-α (*A*) or His-hPPAR-γ (*B*) LBD was incubated with 100 nmol/l [^{3}H]KRP-297 in the presence of a 100-fold excess of various unlabeled competitors. 100% binding indicates the total binding of [^{3}H]KRP-297 in the absence of competitors. BF, bezafibrate; BRL, BRL-49,653; KRP, KRP-297; PIO, pioglitazone; TRO, troglitazone; WY, WY-14,643.

reported a fibrate derivative, GW-2,331, that binds directly to PPAR-α and PPAR-γ based on a binding assay, although the biological effects of that compound were not described.

Obese rats exhibited reduced lipid oxidation, increased lipogenesis, and TG accumulation in the liver, which is consistent with the findings of previous reports (15,16). Decreased plasma β-hydroxybutyric acid levels in obese rats also support a reduced ketogenesis. Thus, these abnormalities of lipid metabolism in the liver might contribute to the development of hyperlipidemia. A major finding of this study is that reduced lipid oxidation in liver was restored in KRP-297-treated obese rats, whereas this effect was not observed in BRL-49,653-treated obese rats. Concomitantly, KRP-297, unlike BRL-49,653, increased mRNA levels and the activity of ACO, which is known to be upregulated by PPAR-α (17), in liver of obese rats. In addition, KRP-297 activated rat PPAR-α in a transactivation assay and increased ACO mRNA levels in primary rat hepatocytes, indicating that the in vivo effect of KRP-297 on ACO is due to a direct action via PPAR-α activation. Simultaneously, these results suggest that restoration of reduced lipid oxidation by KRP-297 in the liver may be mediated by induction of hepatic PPAR-α–upregulated genes encoding key enzymes in the lipid oxidation pathway (18,19).

BRL-49,653 inhibited TG accumulation in liver of obese rats. BRL-49,653 tended to restore enhanced lipogenesis in the liver, although this effect was not significant. These effects may be mediated by lowering of the plasma levels of glucose and insulin involved in the induction of lipogenic enzymes in the liver (29). On the other hand, KRP-297 showed greater inhibition of elevated lipogenesis and TG accumulation in the liver than BRL-49,653. Stimulation of lipid oxidation may diminish the levels of intracellular lipid intermediates available for lipogenesis. Therefore, the inhibitory effect of KRP-297 on lipogenesis and TG accumulation in the liver may be explained, at least in part, by restoration of the reduced lipid oxidation in the liver, in addition to lowering the levels of plasma glucose and insulin.

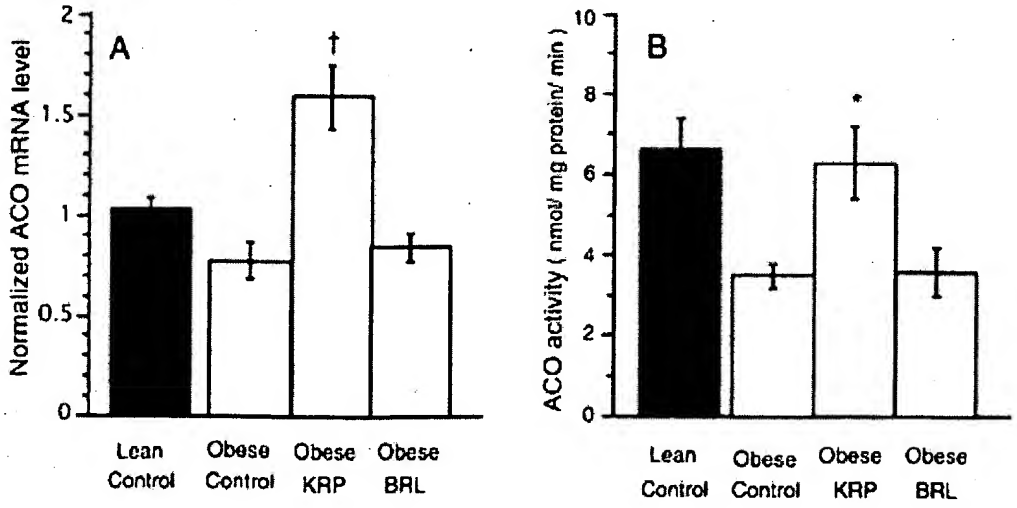


FIG. 6. Hepatic ACO mRNA expression and activity in obese rats. mRNA (*A*) and activity (*B*) of ACO in light mitochondrial fraction of the liver were determined as described in METHODS. Amount of ACO mRNA was normalized by β-actin mRNA. Data are means ± SE. *$P < 0.05$ vs. obese control rats; †$P < 0.01$ vs. obese control rats. BRL, BRL-49,653; KRP, KRP-297.

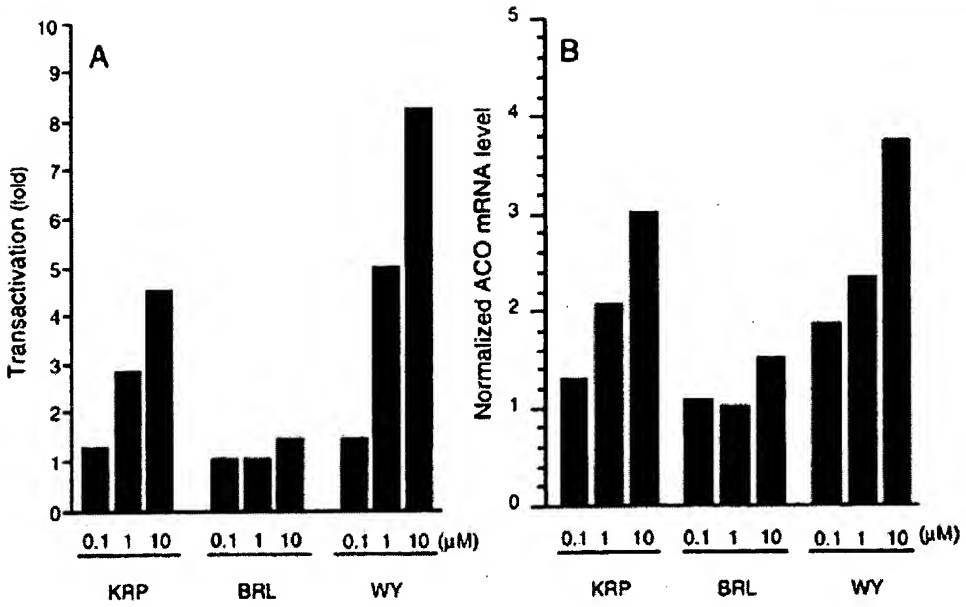


FIG. 7. Transactivation for rat PPAR-α and expression of ACO mRNA in primary rat hepatocytes. *A*: ACO-PPREx3-luciferase reporter plasmid and β-gal plasmid were cotransfected with or without rat PPAR-α plasmid, and cells were then cultured in the presence of indicated concentrations of KRP-297 (KRP), BRL-49,653 (BRL), WY-14,643 (WY), or vehicle (0.1% DMSO) control. Each luciferase activity was normalized by β-gal activity, and each activity in the presence of rat PPAR-α was compared with that in the absence of rat PPAR-α. Each data point represents fold increase in activation over vehicle control. *B*: Hepatocytes were treated for 24 h with the indicated concentrations of KRP-297, BRL-49,653, and WY-14,643. Each ACO mRNA level normalized by β-actin mRNA level is represented as the ratio of vehicle control.

PPAR-α activators are known to promote hepatomegaly and hepatic peroxisome proliferation in rodents (30), although this mechanism is still unclear. Nevertheless, even an excessive dosage (300 mg/kg) of KRP-297, unlike bezafibrate, failed to elicit hepatomegaly and hepatic peroxisome proliferation in rats (S. Koga, K.M., unpublished observations). In fact, KRP-297 tended to decrease the liver weight in obese rats. Thus, PPAR-α activation by KRP-297 does not appear to be associated with promotion of hepatomegaly and hepatic peroxisome proliferation. Simultaneously, PPAR-α activation without peroxisome proliferation may be sufficient to stimulate lipid oxidation and to show hypolipidemic actions in vivo. As suggested in this study, we interpret these results to indicate that PPAR-α activation may be essential, but not sufficient, for the development of hepatomegaly and peroxisome proliferation in the liver (31).

TZD derivatives improve hyperglycemia, hyperinsulinemia, and hyperlipidemia in various insulin-resistant animal models. Although this mechanism of action has not been entirely established, several possible actions via PPAR-γ activation in adipose tissue have been proposed (32,33). PPAR-γ activation promotes adipocyte differentiation and gene expression of adipose lipoprotein lipase involved in clearance of circulating lipids (33,34). The plasma glucose, insulin, TG, and free fatty acid levels in treated obese rats were similar despite the fact that KRP-297 was less potent than BRL-49,653 in inducing mRNA expression of the PPAR-γ–upregulated aP2 gene in retroperitoneal adipose tissues. This finding may suggest that a lower level of PPAR-γ activation (adipogenesis) in adipose tissues is needed for KRP-297 than for BRL-49,653 to produce a similar extent of glucose-, insulin-, or lipid-lowering effects in plasma of obese rats. Furthermore, the hypolipidemic effect of KRP-297 may be also mediated by restoration of abnormal lipid metabolism in the liver via PPAR-α activation in addition to several mechanisms via PPAR-γ activation in adipose tissues, which is consistent with the previous report that the combination of fibrate and BRL-49,653 resulted in an additive hypolipidemic action in normal rats (35). Based on the present data, we postulate that PPAR-α activation in the liver may block the development of hyperglycemia and hyperinsulinemia in obese rats through inhibition of lipotoxic effects such as elevated circulating lipids and/or hepatic cellular lipids (11–14).

TABLE 2
Effect of KRP-297 and BRL-49,653 on aP2 expression in adipose tissue of obese rats

Rat	Treatment	aP2 mRNA level (%)
Obese	Vehicle	121 ± 40
Obese	KRP (10 mg/kg)	827 ± 178*
Obese	BRL (10 mg/kg)	1,118 ± 219†
Lean	Vehicle	100

Data are means ± SE. For all groups, $n = 4$. *$P < 0.05$ vs. obese vehicle; †$P < 0.01$ vs. obese vehicle.

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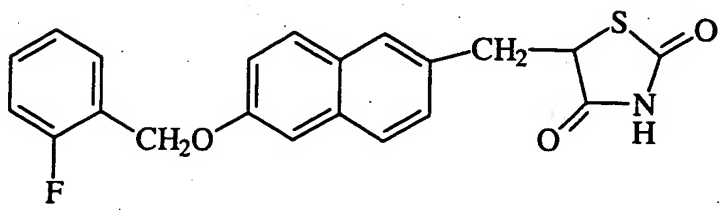
USAN	NETOGLITAZONE
PRONUNCIATION	net oh glit' a zone
THERAPEUTIC CLAIM	antidiabetic; orally-administered insulin action enhancer to lower the plasma glucose in patients with non-insulin dependent diabetes mellitus (Type II)

CHEMICAL NAMES

(1) 2,4-thiazolidinedione, 5-[[6-[(2-fluorophenyl)methoxy]-2-naphthalenyl]methyl]-

(2) (±)-5-[[6-[(*o*-fluorobenzyl)oxy]-2-naphthyl]methyl]-2,4-thiazolidinedione

STRUCTURAL FORMULA



MOLECULAR FORMULA	$C_{21}H_{16}FNO_3S$
MOLECULAR WEIGHT	381.42
TRADEMARK	Unknown as yet
MANUFACTURER	Mitsubishi-Tokyo Pharmaceuticals, Inc.
SPONSOR	R.W. Johnson Pharmaceutical Research Institute
CODE DESIGNATIONS	MCC 555; RWJ-241947
CAS REGISTRY NUMBER	161600-01-7

taken from "2001 Published USAN," at http://www.ama-assn.org/

STATEMENT ON A NONPROPRIETARY NAME ADOPTED BY THE USAN COUNCIL:

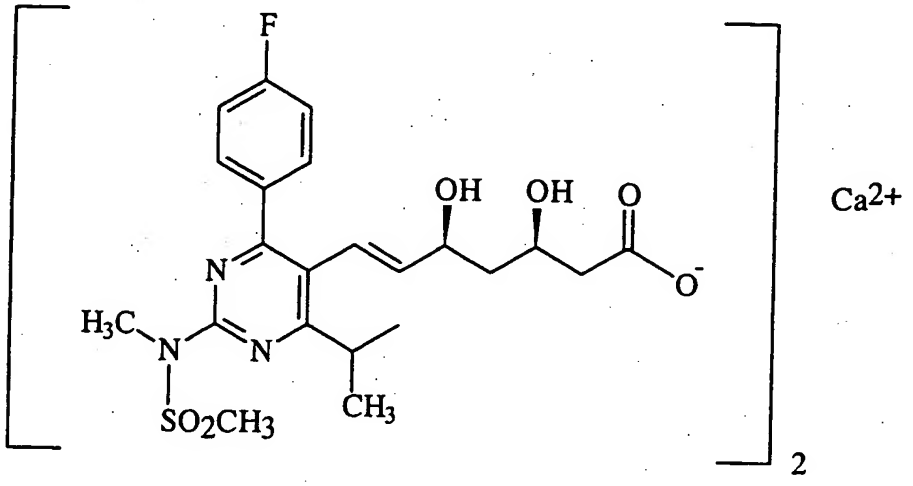
USAN	ROSUVASTATIN CALCIUM
PRONUNCIATION	roe soo′ va sta tin
THERAPEUTIC CLAIM	antihyperlipidemic (HMGCoA reductase inhibitor)

CHEMICAL NAMES

(1) 6-heptenoic acid, 7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-, calcium salt (2:1), (3*R*,5*S*,6*E*)-

(2) [*S*-[(*R**,*S**-(*E*)]]- 7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl) amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid, calcium salt (2:1)

STRUCTURAL FORMULA



MOLECULAR FORMULA	$2C_{22}H_{27}FN_3O6S \cdot Ca$
MOLECULAR WEIGHT	1001.14
TRADEMARK	Unknown as yet
MANUFACTURER	AstraZeneca Pharmaceuticals LP
CODE DESIGNATIONS	ZD4522; S-4522
CAS REGISTRY NUMBER	147098-20-2

Taken from "2002 Published USAN," at http://www.ama-assn.org/